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Studies on I) Dry Matter and Nitrogen Disappearance of Six Soybean Protein Products In Situ and II) Contamination of In Situ Dry Matter and Nitrogen Disappearance with Acid Detergent Fiber

James Coomer

STUDIES ON I) DRY MATTER AND NITROGEN DISAPPEARANCE OF SIX SOYBEAN PROTEIN PRODUCTS *IN SITU* AND II) CONTAMINATION OF *IN SITU* DRY MATTER AND NITROGEN DISAPPEARANCE WITH ACID DETERGENT FIBER

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In experiment I), dry matter disappearance (DMD) and nitrogen disappearance (ND) of raw soybeans (RAW), solvent extracted soybean meal (SBM), heat treated whole soybeans (HT), mechanically extracted soybean meal (MEX), dry extruded soybeans (DEX), and wet extruded soybeans (TEX), were studied in situ for times of 3,6,12 and 24 h of rumen exposure. Five gram, air dry, samples were suspended in the rumen of a lactating Holstein cow fed a total mixed ration twice daily. The percent DMD for 24 h was as follows: RAW-85.9; SBM-56.6; HT-39.0; MEX-40.2; DEX-28.0; TEX-43.3. The greatest DMD was observed with RAW and was greater than all others ($P<.01$), followed by SBM which was significantly greater than all but RAW ($P<.01$). DEX presented the lowest DMD when compared to all others ($P<.01$). Percent ND values for 24 h for the soy products were: RAW-90.8; SBM-47.0; HT-32.7; MEX-25.7; DEX-16.5; TEX-23.0. The ND for RAW was significantly greater ($P<.01$) than all others, while the ND for SBM was similar ($P>.01$) to HT but greater ($P<.01$) than MEX, DEX and TEX. ND for HT, MEX, DEX and TEX were similar ($P>.01$). Significant differences were observed in DMD and ND of various soybean products. As expected, a high degree of degradation and ND was observed with raw soybeans. The application of heat decreased DMD and ND in SBM and application of greater heat (HT, MEX and DEX) and application of heat with moisture (TEX) resulted in products with lower DMD and lower ND.

In experiment II) wheat straw acid detergent fiber (ADF) was subjected to *in situ* DMD and ND studies. Effects of time (6-12-24 h) and sample weight (1-2-3 grams) were evaluated. A lactating Holstein cow being fed a total mixed ration was used. ADF dry matter (DM) weights (after incubation) expressed as a % of the original sample, were as follows: one gram; 101, 110 and 136; two grams; 99, 106 and 110; and three grams; 97, 110 and 114 for 6, 12 and 24 h respectively. The ADF DM weights of the one and two gram samples were significantly higher ($P<.05$) for 12 and 24 h than 6 h. When sample sizes were combined for each time, comparisons found 24 h to be significantly higher ($P<.01$) than 6 h. The DM changes were also reflected and magnified in the % N changes in the ADF residues. The amount of N of the one gram ADF samples increased 32% after 6 h, 122% after 12 h and 287% after 24 h (24>12>6-$P<.01$), and for two grams 29%-6 h, 97%-12 h and 117%-24 h (24>12>6-$P<.05$), and for three grams 34%-6 h, 140%-12 h and 142%-24 h (24 and 12>6-$P<.01$). Potential problems with DM and N contamination of ADF residue with *in situ* studies were demonstrated with small increases in DM weights and larger increases in N content.

Introduction

The level of protein in livestock rations is extremely important and should directly or indirectly supply the amino acids needed by the animal at the tissue level. Ruminants have a certain need for pre-formed essential amino acids, as do non-ruminants, at the tissue level. These metabolizable amino acids can arise from two sources 1) microbial synthesis and 2) rumen escape dietary protein (Mantysaari et al., 1989). In ruminants, non-protein nitrogen and much of the dietary protein is converted to ammonia (NH_3) by the rumen microbial population. The NH_3 is then used for rumen microbial synthesis. The microbes, upon reaching the small intestine, supply amino acids that can support a low level of production (Satter et al., 1977). High producing ruminants have a higher protein (amino acid) requirement than can be met by simple microbial protein synthesis, dictating that a portion of dietary protein fed to the ruminants should be resistant to rumen bacterial breakdown and should reach the absorption site as intact (by-pass) but digestible (available) protein (Satter et al., 1977).

When formulating a ration of 16-18% crude protein for high producing ruminants, the ingredients must contain some by-pass protein to complement that resulting from microbial synthesis (Satter et al., 1977). Because of the balanced need for essential amino acids at the site of absorption, there is concern about the amino acid profile of the protein reaching the small intestine. Many proteins will undergo some changes in their amino acid composition during rumen fermentation; if these changes cause the

amino acid ratio of the absorbed protein to be out of balance with the needs of the animal, both the productivity and protein efficiency will decrease (Mantysaari, 1989). When formulating a ration for high producing ruminants, one faces the problem of supplying a source of by-pass protein that will also maintain an amino acid balance, after rumen fermentation, close to the amino acid needs of the animal at the abomasum and small intestine level.

The use of *in situ* evaluation of protein by-pass is one of the most used lab techniques. However, as noted, the natural rumen microbial fermentation is a major pathway in supplying the needed amino acids. It should be noted that a considerable number of microbes may attach to the feedstuff residue (Nocek, 1988), thus presenting problems of microbial (N-protein) contamination which may present problems of interpretation of *in situ* studies.

The purposes of these studies are twofold: 1) to determine *in situ* dry matter disappearance (DMD) and nitrogen disappearance (ND) of raw soybeans (RAW), solvent extracted soybean meal (SBM), mechanically extracted soybean meal (MEX), heat treated whole soybeans (HT), dry extruded soybeans (DEX), and wet extruded soybeans (TEX) to compare the effects of different processing techniques on the amount of by-pass protein in soybeans; and 2) to determine dry matter and nitrogen contamination of rumen *in situ* studies with acid detergent fiber (ADF) to better estimate the true dry matter and nitrogen digestibility of feedstuffs *in situ*.

Nutritional Requirements

Nutritional requirements of animals are constantly changing with variations in level of production, health and activity. The highest nutritional requirements are for high producing animals including fast growing young animals. Young fast growing ruminants are in the process of developing a complicated digestive system. At birth the ruminant is functionally a monogastric but soon develops a digestive system which takes advantage of a symbiotic relationship between it and rumen bacteria and protozoa. These have the ability to break down cellulose and utilize it as an energy source as well as use non-protein nitrogen (NPN) for protein synthesis and growth. The microbial population can then be used as a source of nutrients (protein/AA) to meet the needs of the ruminant. This is what sets the ruminant apart from the monogastric.

Table 1 (NCR, 1984) shows the daily nutritional needs of a large frame, fast growing beef animal and a small frame, mature beef cow and Table 2 (NCR, 1988) shows that of a high milk producing and low producing dairy cow.

Table 1. Nutritional requirements of beef cattle.[1]

Body Weight (Kg)	Daily gain (Kg)	Energy (MCal/day) NEm	NEg	Protein g/day	Ca g/day	P g/day
600[2]	1.8	9.33	10.10	1071	39	26
350[3]	0.0	0.92	N/A	478	12	12

[1] NCR, 1984
[2] Large, frame bull calves and compensating large frame yearling steers
[3] Dry pregnant mature cows - middle third of pregnancy

Table 2. Nutritional requirements of dairy cattle.[1]

Body weight (Kg)	Daily milk (Kg)	Fat (%)	TDN (Kg)	Total CP (g)	Total Ca (g)	Total P (g)
800	40	3.5	17.30	3930	150	96
800	15	3.5	9.78	1955	77	50

[1]NCR, 1988

As Tables 1 and 2 show, the nutritional needs of high producing animals, especially in regard to protein, are much higher than those of the low producing animals. Low producing ruminants can meet their protein needs by conversion of dietary protein or NPN into microbial protein in the rumen, providing sufficient energy and other nutrients are available in the ration. However, the high producing ruminant's need for protein appears to exceed the rumen's ability to produce microbial protein. Therefore, it needs a source of protein that will bypass rumen degradation. This can help supplement the microbial protein (amino acids) at the tissue level. McCullough (1984) estimated that a cow producing 70 pounds of milk per day gets half of her protein requirements from the microbial protein and the other half from by-pass protein.

Protein Requirements

As pointed out earlier, protein requirements differ among animals, and for low producing ruminants the protein needs can be met by microbial protein synthesis. In the rumen, bacteria and protozoa breakdown both NPN and feed protein into ammonia (NH_3). This NH_3 is then incorporated into microbial protein which passes into the lower part of the gastro intestinal tract where it is broken down by enzymes and utilized as a

source of amino acids (AA). Rumen microbes are an excellent source of protein. Hespell and Bryant (1979) reported that ruminal microbes varied in crude protein content, but during rapid growth, averaged 65% CP. Even though microbial protein is of a high quality, there does seem to be an upper limit to the capacity of the rumen microflora to utilize NH_3 and produce microbial protein, assuming sufficient energy and other nutrients. Beyond this limit NH_3 may begin to accumulate, be absorbed, detoxified and eventually excreted as urea in the urine (Bull et al., 1977). Where the upper limit for NH_3 utilization occurs is vague, Satter and Roffler (1975) postulated that NH_3 accumulation may occur at a rumen ammonia-nitrogen (NH_3-N) concentration of approximately 5 mg/100 ml. This may occur when diets contain 12-13% dietary crude protein (CP). However, Bartley (1976) refutes this. Using data from Helmer et al. (1970) Bartley shows that NPN can be utilized for microbial synthesis at NH_3-N levels as high as 124 mg/100 ml, and in vivo studies by Stiles et al. (1970) support these findings.

High producing ruminants, such as high producing dairy cows and fast growing feedlot animals, have protein or AA needs, at the tissue level, that exceed the capacity of the rumen to produce microbial protein. These animals require a protein source that is partially resistant to rumen microbial degradation (Satter et al., 1977). These animals also need a source of degradable protein or utilizable NPN for conversion into NH_3 in the rumen to maintain the still considerable rumen microbial synthesis. The protein that is not degraded in the rumen is referred to as by-pass protein and should reach the lower parts of the gastrointestinal tract as intact protein or AA, and still in a form that can be broken down by the enzymes of the abomasum and small intestines. The total AA needs

of the ruminant are met by both microbial protein and by-pass protein being digested at the level of the abomasum and small intestine.

By-pass Protein

The amount of microbial and intact dietary protein available post ruminally varies and can be manipulated (Polan, 1988). One way of manipulating the amount of by-pass protein reaching the abomasum is to vary the feedstuffs used in the ration. The amount of by-pass protein in different feedstuffs vary (Table 3).

Table 3. Rumen in situ crude protein disappearance of selected feedstuffs after 24 h incubation.

Item	% Crude Protein Disappearance			
	References[1,2,3,4]			
	1	2	3	4
Raw Feeds				
Corn	61.4			50.0
Oats	86.3			77.5
Whole Cottonseeds		92.3		
Raw Soybeans			92.6	
Processed Feeds				
Cottonseed Meal	54.2			58.3
Extruded Cottonseeds		73.8		
Soybean Meal	67.0	92.2	66.1	61.5
By-product Feeds				
Corn Gluten Feed	74.8			19.8
Brewers Dried Grains	52.1			
Distillers Dried Grains	71.0		14.6	

[1] Erdman et al., 1967
[2] Stutts et al., 1988
[3] Fotouhi, 1987
[4] Barrio et al., 1986

There are differences in by-pass protein content of different feedstuffs, but there are also differences of by-pass protein of the same feedstuff, i.e., by-product feeds. This variation for by-product feeds can be attributed to different processing conditions. Therefore, when using by-product feeds in a ration, composition and digestibility and *in situ* disappearance should be determined. Another example of the variation in by-pass protein of different feedstuffs can be seen in Table 4.

Table 4. Daily nitrogen (N) intake and nitrogen flow to the duodenum of lactating cows fed diets containing either soybean meal, corn gluten meal, wet brewers grains or dried distillers grains as a protein supplement.[1]

Item	SBM[2]	CGM[2]	WBG[2]	DDG[2]
Nitrogen intake, g/d	396	412	333	404
Flow to duodenum, g/d	462[a]	576[b]	475[a]	538[b]
Total NAN[2]	446[a]	559[b]	461[a]	525[b]
Bacterial N	328	333	302	308
Dietary N	118[a]	226[b]	160[a,b]	217[b]

[1]From Santos, Stern and Satter (1984).
[2]Abbreviations: SBM, soybean meal; CGM, corn gluten meal; WBG, wet brewers' grains; DDG, dried distillers' grains; NAN, non-NH_3-N.
[a,b]Means in the same row with different superscripts are different ($P < .05$).

In Table 4 the flow of dietary N into the duodenum is different for these feeds, varying from 118 g/d to 226 g/d while bacterial N flow remained relatively constant, varying from 302 g/d to 333 g/d. The variation in availability of by-pass protein is considerable between by-product feeds. However, we may see similar variations within the same feedstuff due to different processing techniques.

There are many types of processing that can be used on feedstuffs, including heat treating, extruding, expeller processing, pelleting and

jet-sploding as well as treatments with formaldehyde or calcium lignosulfate. These processes or treatments can alter the degradability of the dry matter and protein in the rumen as well as the degradability in the whole tract. Heat treating tends to decrease both DM and N degradability in the rumen (Stutts et al., 1988, Lubbadeh, 1986, and Arieli et al., 1989). Arieli et al. (1989), while observing reduced rumen degradability, also noted heat treating (130+°C) of whole cottonseeds improved whole tract digestibility. There are mixed results on the effect of extrusion on ruminal degradation of DM and N. Stutts et al. (1988) showed extrusion, at temperatures above 146°C, to reduce rumen degradation of whole cottonseeds while Deacon et al. (1988) found extrusion to have no effect on the rumen degradation of soybean meal and canola meal. These differences could be due to different extrusion temperatures or conditions. Broderick (1986) showed expeller processing of soybeans reduced rumen degradation of DM and N as compared to solvent extracted soybean meal. Broderick and Craig (1980) also showed expeller processing of cottonseed meal reduced ruminal degradation. Jet-sploding (jet-sploder®, California Pellet Mill Co., Crawfordsville, Indiana) uses a high temperature (315°C) for a short period of time to cook the feedstuff. This type of processing has been proven (Deacon et al., 1988) to dramatically reduce rumen degradability of whole canola seed. By-pass protein can be altered but there must be concern about the amino acid balance and availability of the protein reaching the small intestine.

Amino Acid Balance

In ruminants, one half or more of the total amino acid needs are met by microbial protein (Table 4). Ruminal microbes contain all essential amino acids (AA), and the AA profile of ruminal microbes tends to stay

relatively constant. Table 5 presents the AA composition of milk, cattle tissue, rumen microbes, corn grain and soybeans.

Table 5. Amino acid composition of proteins of cattle tissue, rumen microbes, corn grain and soybean expressed as a percent of protein.

Amino Acid	Cattle Tissue[1]	Milk[1]	Rumen Microbes[1]	Corn Grain[2]	Soybean[2]
Methionine	2.7	2.7	2.68	2.0	1.3
Lysine	8.2	8.3	10.46	2.5	6.3
Histidine	3.0	2.7	2.69	2.0	2.4
Phenylalanine	4.6	5.3	5.16	NR	NR
Tryptophan	1.2	1.4	1.63	1.0	1.3
Threonine	4.6	4.6	5.59	4.0	3.7
Leucine	7.2	10.0	7.51	11.1	7.4
Isoleucine	5.5	6.0	5.88	5.1	5.5
Valine	5.2	6.7	6.16	4.0	5.2
Arginine	6.8	3.7	6.96	NR	NR

[1]Mantysaari et al., 1989
[2]Van Soest, 1982
NR - not reported

Comparing the AA composition of rumen microbes with milk and cattle tissue indicates there should not be any limiting AA for growth and milk production. Corn grain and soybean are both low in methionine, lysine and histidine. Schwab (1976) reported that lysine and methionine are the first and second limiting or co-limiting AA for lactating dairy cattle on low protein, high corn diets. Table 6 (Satter, 1986) illustrates how soybean meal and dried distillers grains may supply these two AA to the site of absorption (i.e. g AA escaping).

Soybean meal is high in lysine but low in methionine and distillers dried grain is high in methionine, and is low in lysine. But the percent by-pass of DDG is higher than that of SBM, thereby altering the total amount of AA that will reach the tissue level. A diet with SBM as the

Table 6. Expected supply of lysine and methionine to the intestine when soybean meal and distillers dried grain are fed.[1]

Amino Acid	Source	(g) AA in 1 Kg Protein	Fraction of Protein Escaping	(g) AA Escaping
Lysine	SBM[2]	68.2	.30	20.5
	DDG[2]	30.3	.55	16.7
Methionine	SBM	11.4	.30	3.4
	DDG	16.2	.55	8.9

[1]Satter, 1986
[2]Abbreviations: SBM, soybean meal; DDG, distillers dried grain

only protein supplement would tend to be limited by methionine while a diet with DDG as the only protein source would be limited by lysine; however, when the two are fed together, they should provide a more balanced supply of dietary lysine and methionine to the abomasum and small intestine.

Ruminants, like non-ruminants, have essential AA requirements at the site of absorption. Ruminants respond to a balanced supply of AA reaching the site of absorption more than they respond to the amount of crude protein consumed. For this reason the quality (AA balance) of by-pass protein may be more important than quantity. There are differences between the AA profile of the protein portions that escape rumen degradation and the AA profile of the protein originally fed (McGregor, 1978). Table 7 presents the differences in AA profiles of SBM, high moisture shell corn, alfalfa (fresh) and fish meal, before and after rumen incubation.

There was a reduction in the quantity (percent) of lysine in all feeds following rumen incubation, while there was a slight increase in the quantity of methionine in all feeds except alfalfa. ARG, GLU and TYR showed varied results between feeds following rumen incubation. GLU

Table 7. Amino acid profile of soybean meal, high moisture shell corn, alfalfa and fish meal (F) and their residues (R) after rumen incubation.[1]

Amino Acid	Soybean Meal		High Moisture Shell Corn		Alfalfa (fresh)		Fish Meal	
	F[2]	R[3]	F	R	F	R	F	R
ARG[4]	8.49	5.57	2.04	2.08	4.78	4.13	7.91	7.19
GLU	18.47	10.30	11.17	19.33	7.67	7.56	11.56	11.30
LYS	7.21	5.88	1.76	1.31	4.92	4.62	8.30	7.13
MET	1.57	1.61	.61	.64	1.21	1.19	2.61	2.84
TYR	3.33	2.55	.52	.97	2.35	1.84	3.18	3.63

[1]Mantysaari et al., 1989
[2]g/100 g feed protein
[3]g/100 g undegraded feed protein
[4]Abbreviations: ARG, arginine; GLU, glutamine; LYS, lysine; MET, methionine; TYR, tyrosine

increased in the corn following incubation and decreased in the SBM while staying about the same in the alfalfa and fish meal. The value of a protein supplement as a source of by-pass protein should be determined by the amount of balanced AA left in the portion escaping rumen degradation. There are several methods for estimating the values of a feed as a by-pass protein supplement.

Protein Disappearance In Situ

There are three main methods of estimating rumen N degradability of a feedstuff; they are in vivo, in vitro (N solubility) and the in situ (nylon bag) techniques. The in vivo technique gives the most accurate values for rumen degradability, but it has major draw backs. One negative point is the large amount of time it takes to get degradability values for a single feed, sometimes weeks, and it also usually requires more than one animal with multiple canulas. These reasons, along with the large amounts

of feed and feces that must be handled, as well as the cost prevent the in vivo technique from being very popular.

The in vitro N solubility technique is very popular but generally gives unreliable results. The in vitro method allows several samples to be tested in a short period of time, with a small sample of each feed, and no animals needed. The problems with this method are the large variation involved in the technique used by different researchers as well as the variation found within samples. Though it is not a good method to compare several different feedstuffs, it can be used to rank the degradability of feedstuffs in vivo.

The in situ, or nylon bag, method of determining N disappearance is the most popular or used method of estimating N disappearance or rumen degradability of a feedstuff. The in situ method involves placing samples of a feedstuff in nylon bags and suspending the bags in the rumen of a fistulated animal and then removing the bags at given times and determining degradability by comparing the amount of DM or N remaining in the bag with the amount that was originally placed in the bag. Mehrez and Orskov (1977) found this method to provide a relatively accurate measurement of the extent and rate of nitrogen disappearance (ND) and dry matter disappearance (DMD).

There are many factors that affect in situ degradability estimates. The feed particles in the bags are not subjected to the same action as the rumen content, i.e., regurgitation, remastication and remixing (Tamminga, 1979). Pore size and uniformity of the material used can greatly affect DMD estimates (Heidker and Behnke). Bag size, or more specifically amount (g) of sample per unit of surface area, can cause variation. The best results have been obtained when 28 to 32 mm^2 of surface area were allowed for each mg of sample (Nocek et al., 1979 and Rodriquez,

1968). Mehrez nad Orskov (1977) determined that a 17 x 9 cm bag was sufficient for testing 5 g samples of a feedstuff and getting acceptable results. Weighted bag holders which hold the bags in an extended position for more uniform contact with rumen contents have been used with good results (Nocek et al., 1979 and Van Hellen and Ellis, 1977). Another possible problem of the *in situ* method may be microbial contamination of the residue. A complete discussion of this topic will appear later.

Although many factors affect the DMD and ND of the *in situ* method, it still provides the best estimate of degradability *in vivo* and allows the degradability of several feedstuffs to be determined concurrently so better comparisons can be made. The *in situ* method can be done relatively quickly with minimal labor and the use of only a few animals. Comparison of 118 feed samples *in vivo*, *in vitro* and *in situ* by Madsen and Hvelplund (1985) demonstrated that the *in situ* method gave the best estimate of the *in vivo* degradability at a passage rate of eight percent. However the nylon bag technique may under-or over-estimate degradability for feedstuffs depending on their protein degradability (Erdman et al., 1987; Stern and Satter, 1984).

Microbial Contamination

The potential problems of microbial contamination of rumen *in situ* study residues is clearly defined by Nocek (1988). "Because of the intimate contact of test feed particles with ruminal microflora, potential contamination with microbial constituents is an inherent obstacle and source of variation associated with estimating true nutrient digestibility of feeds by the *in situ* technique." This problem is not new. Even with *in vivo* digestion studies the differentiation between true and apparent digestibility is always a concern. Mason (1969) studied the distribution

and origin of nitrogen in sheep feces. He concluded that 60-80% of the non-dietary nitrogen was associated with bacterial matter and that most of the bacterial N originated in the rumen. Mason's studies also indicated that only 10-40% of the N in feces was undigested dietary N.

Other researchers such as Akin and Amos (1975), by means of transmission electron microscope and scanning electron microscope, have demonstrated the attachment of rumen bacteria to plant material (both grain and forage). More recently, contamination studies have been conducted using a radioactive sulfur isotope (S^{35}) as well as diaminopimelic acid (DAPA), which is an AA found in rumen bacterial protein, as microbial markers. Estimations of contamination are quite variable.

Nocek (1987) observed a 5% contamination of ground (5 mm screen) dried shelled corn after 16 h and a 50% contamination of rolled corn after 40 h ruminal incubation. Fourteen to 19% contamination of soybean meal (SBM) from 6-24 h was also observed by Nocek (1985). However Blair and Cummins (1983) working with dehydrated alfalfa and SBM, and using S^{35} in an in vitro/dacron bag study, observed 4-12% of the residual nitrogen of the dehydrated alfalfa to be bacterial while, with the SBM they observed up to 41% contamination. This does not agree with Nocek (1985) but does agree with the 9-18% contamination of alfalfa hay for times of 6-24 h observed by Mathers and Aitchison (1981). Nocek and Grant (1987) found 25-44% contamination with alfalfa hay incubated for times of 6-12-24 h.

Interpretation of in situ studies is still difficult. For example, Rooke, Greife and Armstrong (1984), working with S^{35} in silage in situ studies, found significant increases of S^{35} in the residues (up to 386 gm/Kg residual N) but concluded that in their study this would not

markedly alter the digestion values obtained. In other studies with forages 20-70% of the residual N was found to be of microbial nature (Nocek and Grant, 1987) and in many cases this value increased to near 100% by 100 h of incubation.

In summary, there is a tendency for forages to present greater degrees of microbial contamination (though this was not consistent). Most authors observed low percent contamination of SBM (10-20%) though Blair and Cummins (1983) did report up to 41%. Nocek (1985) demonstrated no significant difference in rate coefficients of ND of SBM with or without correction; however, the elevation of the slope was 8.1% units lower for the corrected rate. The conclusion must be, that microbial contamination is significant as we look at protein and especially when we look at AA content of the residues. As we look at the AA composition of rumen microbial protein, it is about 10.5% lysine where corn and soybean protein are about 2.5 and 6.3% lysine respectively (Table 5). Therefore, microbial contamination may cause an under-estimation of the degradation of lysine and an over-estimation of the degradation of other AA.

Materials and Methods

Experiment I

Dry matter (DM) and nitrogen (N) disappearance of raw soybeans (RAW), solvent extracted soybean meal (SBM), mechanically extracted soybean meal (MEX), heat treated whole soybeans (HT), dry extruded soybeans (DEX), and wet extruded soybeans (TEX) were determined for rumen exposure times of 3,6,12 and 24 hours (h). These six soybean protein products had a wide range of processing from none (RAW) to extensive (TEX). The RAW were processed in no way other than being ground for the trial and HT received only a dry air heat treatment in a rolling drum at about 150°C (Ensminger, 1985) and was ground for the trial. Processing of SBM and MEX included extraction of lipids by solvent or by mechanical pressure. Some heat was added to the SBM after extraction to remove the solvent, and there was considerable heating (163°C) of the MEX due to the pressure used to extract the lipids. The SBM was ground before extraction, and MEX was ground after extraction. DEX were processed by extrusion without addition of heat or moisture (Smith, 1985). Some cooking does take place due to the friction of the soybeans being forced through a screw apparatus with progressively narrower pitch and forced through a die (Smith, 1985). The TEX received the most processing, starting with grinding and solvent extraction of lipids. The meal is then ground into a fine powder, and moisture is added in the form of steam and the mixture is formed into a dough-like consistency at temperatures of 82 to 99°C (Smith, 1985). This mixture is then extruded and heat is added during extrusion to raise the

temperature to 110 to 240°C for about 10-20 seconds; then the mixture is forced through a die (Smith, 1985). The TEX is sometimes referred to as textured soyflour and is used as a meat extender. Table 8 shows a complete analysis of each product for comparison. SBM, MEX and DEX were tested in their commercial particle size while RAW, HT and TEX were ground in a Wiley mill through a 2 mm screen to obtain a similar particle size to the other three products.

Table 8. Analysis of soybean protein products giving values for crude protein, crude fiber, ether extract, nitrogen free extract and ash on a dry matter basis.

Feed	Crude Protein	Crude Fiber	Ether Extract	Nitrogen Free Extract	Ash
	----------	----------	%	----------	
Raw Soybeans	42.0	6.3	17.0	32.4	2.3
Solvent Extracted	51.5	3.7	0.9	38.5	5.4
Heat Treated	40.5	4.3	18.8	34.3	2.1
Mechanically Extracted	47.0	5.0	7.4	35.2	5.4
Dry Extruded	35.0	4.3	17.9	37.4	5.4
Wet Extruded	55.0	1.0	1.0	37.8	5.2

Five (5) gram (air dried) samples of each feedstuff were placed in nylon bags, and the bags were tied with a nylon string, i.e., dental floss. The bags were constructed of Nitex® nylon bolting cloth and had an average pore size of 44 microns (Heidker and Behnke). The bags were constructed by folding a 76.2 mm x 241.3 mm piece of cloth in half lengthwise and stitching the sides with a small straight, continuous stitch, making rounded corners. An overcast stitch was used outside the seam line to prevent the edge of the material from unraveling (Heidker and Behnke).

The bagholders were constructed of clear acrylic plastic (Heidker and Behnke) and could hold eight bags. The bags were suspended in the rumen of a fistulated Holstein cow for either 3,6,12 or 24 h. All samples were placed in the rumen at 6:00 h and removed at the appropriate times. All samples of an experimental period were placed in the rumen at the same time and removed at the same time with samples of different experimental periods placed in the rumen on consecutive days. Two bag holders were used for each experimental period. Each experimental period had triplicate bags of each product except the 24 h period for RAW which had nine bags. The nine bags were randomly placed into three groups of three bags, and each group's contents were combined to obtain an adequate sample size for nitrogen analysis. After removal from the rumen, the bags were rinsed under running tap water at the barn until the runoff water was clear. The bags were rinsed with running tap water a second time, for five minutes, in the lab. The bags were dried in a forced air oven at 105°C then weighed for dry matter analysis and stored for nitrogen analysis.

The fistulated cow was a lactating pregnant Holstein weighing about 600 kg. The cow was fed a diet of approximately 45 kg corn silage, 2.2 kg alfalfa haylage and 1.5 kg grain in a total mixed ration twice daily at approximately 6:00 h and 18:00 h and also received about one half kg of a 16% CP dairy pellet in the milking barn twice daily (DM).

Nitrogen (N) content of the feed residues was determined by a Leco CHN-600 Carbon-Hydrogen-Nitrogen determinator (Leco Corporation, St. Joseph, Michigan). The N percent was determined on samples of approximately 150 mg from each bag. The data were subjected to a randomized complete block design analysis of variance technique. Duncans New Multiple-Range test was used to determine the significant mean differences (Steel and Torrie, 1980).

Experiment II

Dry matter and nitrogen contamination of acid detergent fiber (ADF) was determined by first grinding wheat straw in a Wiley mill through a 2 mm screen. The straw was then subjected to acid detergent digestion (Goering and Van Soest, 1970) to obtain ADF residue. Air dry samples of one, two and three grams were placed in nylon bags, and the bags were tied with nylon string, i.e., dental floss. One, two and three gram samples were used to correspond with DM residues of previous in situ studies. The bags were suspended in the rumen of a fistulated Holstein cow for either 6,12 or 24 h. All samples of a given experimental period were placed in the rumen at 6:00 h and removed at the appropriate time, and samples of other experimental periods were placed in the rumen on consecutive days. Two bag holders were used for each experimental period to hold the triplicate bags of each sample size. After removal from the rumen the bags were rinsed under running tap water at the barn until the runoff water was clear. The bags were rinsed with running tap water a second time, for five minutes in the lab. The bags were dried in a forced air oven at 105°C and weighed for dry matter analysis and stored for N analysis.

The fistulated cow was a lactating, pregnant Holstein weighing about 600 kg. The cow was fed a diet of approximately 45 kg corn silage, 2.2 kg alfalfa haylage and 1.5 kg grain in a total mixed ration twice daily at about 6:00 h and 18:00 h and also received about one half kg of a 16% CP dairy pellet in the milking barn twice daily (Dry basis).

Nitrogen content of the ADF residue was determined by testing approximately 150 mg from each bag for percent N in a Leco CHN-600 Carbon-Hydrogen-Nitrogen determinator (Leco Corporation, St. Joseph, Michigan). The data were subjected to a completely random design analysis of variance technique. Dumcans New Multiple-Range test was used to determine the significant mean differences (Steel and Torrie, 1980).

Results and Discussion

Dry Matter and Nitrogen Disappearance In Situ

Many factors affect *in situ* dry matter and nitrogen disappearance from nylon bags. Each of these factors can have an adverse effect on the rate and degree of dry matter disappearance (DMD) and nitrogen disappearance (ND). The DMD for the six soybean protein products was calculated at 3,6,12 and 24 h of rumen incubation and is presented in Table 9 and plotted in Figure 1.

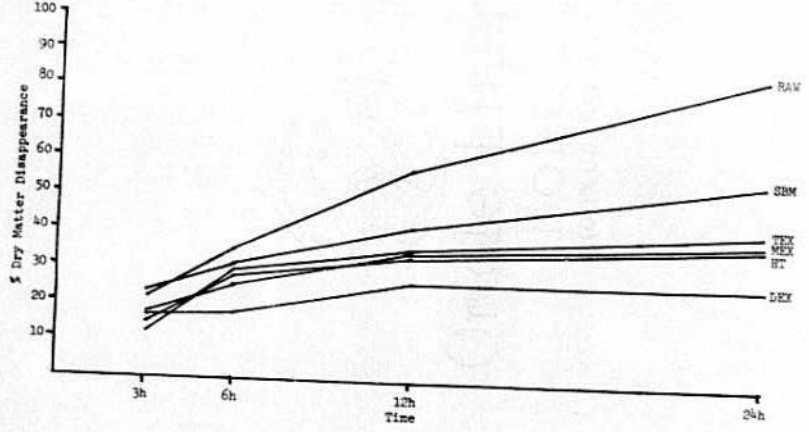
Table 9. Percent *in situ* dry matter disappearance of dry extruded soybeans, heat treated soybeans, mechanically extracted soybean meal, wet extruded soybeans, solvent extracted soybean meal, and raw soybeans at 3,6,12 and 24 h of rumen exposure.

Feed	Time			
	3	6	12	24
	----------	----------	% ----------	----------
Dry Extruded	$15.6^{a,w,x}$	$18.2^{a,w}$	$27.3^{b,w}$	$28.0^{b,w}$
Heat Treated	$14.4^{a,w}$	$28.5^{b,x,y}$	$34.5^{b,c,x}$	$39.0^{c,x}$
Mechanically Extracted	$16.6^{a,w,x,y}$	$25.9^{b,x}$	$35.0^{c,x}$	$40.2^{c,x}$
Wet Extruded	$12.3^{a,w}$	$29.7^{b,x,y}$	$35.9^{b,x}$	$43.3^{c,x}$
Solvent Extracted	$23.2^{a,y}$	$31.4^{b,x,y}$	$41.9^{c,x}$	$56.6^{d,y}$
Raw Soybeans	$22.1^{a,x,y}$	$35.2^{b,y}$	$58.0^{c,y}$	$85.9^{d,z}$

^{a,b,c,d}means in the same rows with different superscripts are significantly different ($P<.05$).

^{w,x,y,z}means in the same columns with different superscripts are significantly different ($P<.05$).

Figure 1. Percent dry matter disappearance of dry extruded soybeans (DEX), heat treated soybeans (HT), mechanically extracted soybean meal (MEX), wet extruded soybeans (TEX), solvent extracted soybean meal (SBM) and raw soybeans (RAW) for 3,6,12 and 24 hours of rumen exposure.



The DMD of all products increased with increasing rumen exposure. Significant ($P<.05$) increases in DMD were observed from 3 to 6 h for all products except DEX. From 6 to 12 h the DMD of DEX, MEX, SBM and RAW showed significant ($P<.05$) increases. DMD tended to level off for all products, except SBM and RAW, from 12 to 24 h. Only RAW and SBM had significant ($P<.01$) increases in DMD from 12 to 24 h (Appendix Table 7). As expected RAW had the highest DMD ($P<.01$) at 12 and 24 h followed by SBM which was significantly ($P<.01$) higher than DEX, MEX, TEX and HT at 24 h. The DMD for TEX, MEX and HT were similar ($P>.05$) at 24 h while DEX was lower ($P<.01$) than all others.

Dry matter disappearance values in this study were considerably lower than those found in the literature (Lubbadeh, 1986, Ha and Kennelly, 1984, Stutts et al., 1988, Barrio et al., 1986 and Crooker et al., 1986). In many cases they were as much as 50% lower. However the values are comparable with values obtained by Fotouhi (1987).

The DMD of HT was lower ($P<.05$) than SBM and RAW and this agrees with the results of Stutts et al. (1988), Lubbadeh (1986) and Plegge et al. (1985), who also found the heat treating of soybeans to reduce DMD. Arieli et al. (1989) also found heating (130+$^{\circ}$C) of whole cottonseeds to reduce rumen degradability. However, Mir et al. (1984) and Crooker et al. (1986) indicated that heat treating of whole soybeans and SBM, respectively, did not reduce rumen degradability. A possible explanation for these differences in results may be the temperature of heating. Mir et al. (1984) only heated the soybeans to a maximum of 120°C as opposed to Plegge et al. (1985) and Stutts et al. (1988), who heated the soybeans to a temperature of 145 to 149°C.

This study indicated that extrusion, both wet and dry, significantly ($P<.01$) lowered DMD as compared to SBM and RAW. These results are in

agreement with Stutts et al. (1988), who found extrusion of whole cottonseeds reduced rumen degradation. The results disagree with those of Deacon et al. (1988), but Deacon indicated that the temperature of extrusion in his study may not have been high enough to have satisfactorily reduced degradation.

The DMD of MEX was lower ($P<.05$) than that of SBM in this study. This agrees with Broderick (1986) who, while using the same MEX product, also showed a significant reduction in the DMD of mechanically extracted soybean meal as compared to solvent extracted soybean meal. Fotouhi (1987) illustrated a lower ($P<.05$) DMD of SBM as compared to RAW which was similar to the results found in this study.

The nitrogen disappearance (ND) of the six soybean products was also calculated for 3,6,12 and 24 h of rumen exposure and is shown in Table 10. The ND for the products was plotted, and is presented in Figure 2.

The ND for all products increased with increasing rumen exposure time, with the exception of DEX, which showed a slight, non-significant ($P>.05$), reduction in ND from 3 to 6 h. During the same time period DEX had a slight increase in DMD. This may indicate that the reduction in ND may be due to microbial attachment to the feedstuff, which would cause an underestimation of the ND at 6 h. Nocek (1985) reported bacterial attachment to SBM up to 12 h, and Crooker et al. (1986) reported that part of the increase in CP of SBM residues in nylon bags was due to bacterial contamination. Even though ND values increased with increasing exposure times the only significant increases were found with SBM and RAW. From 6 to 12 h RAW had a significant ($P<.05$) increase in ND, while from 12 to 24 h both RAW and SBM had significant ($P<.05$) increases.

Figure 2. Percent nitrogen disappearance of dry extruded soybeans (DEX), wet extruded soybeans (TEX), mechanically extracted soybean meal (MEX), heat treated soybeans (HT), solvent extracted soybean meal (SBM) and raw soybeans (RAW) for 3,6,12 and 24 hours of rumen exposure.

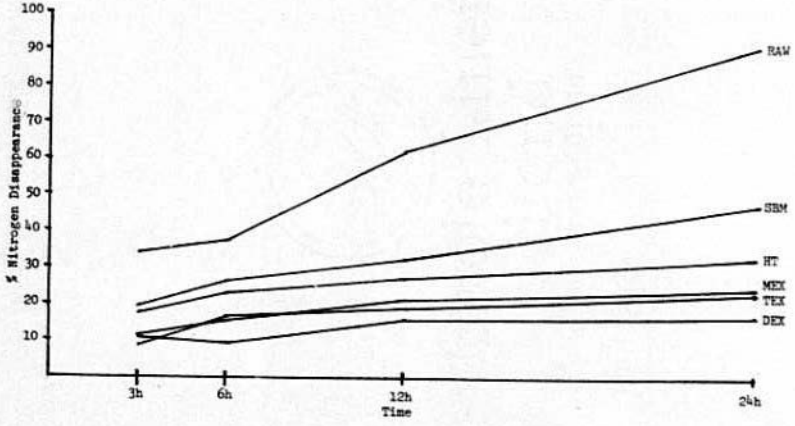


Table 10. Percent in situ nitrogen disappearance of dry extruded soybeans, wet extruded soybeans, mechanically extracted soybean meal, heat treated soybeans, solvent extracted soybean meal and raw soybeans at 3,6,12 and 24 h of rumen exposure.

Feed	Time			
	3	6	12	24
	----------	----------	% ----------	----------
Dry Extruded	$10.6^{a,w}$	$8.7^{a,w}$	$15.3^{a,w}$	$16.5^{a,w}$
Wet Extruded	$7.8^{a,w}$	$16.2^{a,w,x}$	$18.4^{a,w,x}$	$23.0^{a,w,x}$
Mechanically Extracted	$11.0^{a,w}$	$15.6^{a,w,x}$	$20.8^{a,w,x}$	$23.7^{a,w,x}$
Heat Treated	$17.1^{a,w}$	$22.4^{a,w,x}$	$27.8^{a,w,x}$	$32.7^{a,x,y}$
Solvent Extracted	$18.7^{a,w}$	$26.0^{a,x}$	$32.1^{a,x}$	$47.0^{b,y}$
Raw Soybeans	$33.7^{a,x}$	$41.9^{a,y}$	$61.7^{b,y}$	$90.8^{c,z}$

^{a,b,c}means in the same rows with different superscripts are significantly different ($P<.05$).

^{w,x,y,z}means in the same columns with different superscripts are significantly different ($P<.05$).

The lowest ND, at 24 h, was observed with DEX. The ND of DEX was lower ($P<.05$) than HT, SBM and RAW but was similar ($P>.05$) to TEX and MEX. Dry extruded soybeans were followed by TEX with the second lowest ND. Wet extruded soybeans had a similar ($P>.05$) ND to all products except SBM and RAW which had significantly ($P<.05$) higher ND values. The results of this study disagree with those of Deacon et al. (1988) and Blake and Stern (1988), who found extrusion to have no effect on the ND of SBM. However, both studies indicate that the temperature of extrusion may not have been high enough to effectively reduce ruminal degradation. Blake and Stern (1988) did find the extruded SBM to provide

a greater flow of lysine and leucine. Stutts et al. (1988) did, however, find that extrusion of whole cottonseeds reduced ND.

The ND of MEX was similar ($P>.05$) to DEX, TEX and HT and lower ($P<.05$) than SBM and RAW. These results are similar to the results of Broderick (1986). Broderick (1986) compared the same MEX product with commercial SBM using an inhibitor in vitro system and found MEX to provide about 65% more ruminally undegradable protein than SBM. The present study indicated that MEX would provide about 45% more ruminally undegradable protein than SBM.

In this study, HT had a lower ($P<.05$) ND than RAW. This result is contradictory to the results of Mir et al. (1984), who indicated that heat treating of whole soybeans did not reduce rumen degradability. In the study of Mir et al. (1984) the soybeans were heated to 120°C for 20 minutes; therefore, the heat treatment may have not been long enough or at a high enough temperature to effectively reduce the ND. In the present study HT also had a lower, but non-significant ($P>.05$), ND than SBM. The study of Crooker et al. (1986) indicated similar results. However, results from Stutts et al. (1988), Plegge et al. (1985) and Lubbadeh (1986) indicated heating or roasting of SBM did reduce ND or rumen degradability. These studies were heating SBM as opposed to whole soybeans, possibly causing the differences in results.

In comparing SBM and RAW, the ND of SBM was found to be lower ($P<.05$) than that of RAW. Fotouhi (1987) also found SBM to have a lower ND than RAW but Mir et al. (1984) indicated that RAW was only about 15% less degradable than SBM.

The observation of significant increases in DMD of all products over time with only SBM and RAW having significant increases in ND over

time, lead to the second study on possible contamination of in situ studies.

Contamination of ADF In Situ Studies

Because of the results of the first study and an in situ study by Fotouhi (1987), who found some unexplainable results, an experiment was set up to study the extent of microbial contamination of in situ studies. In this study acid detergent fiber (ADF) obtained from wheat straw was used as a substrate. The ADF was chosen because of the ease of obtaining the substrate and its relatively low digestibility. Van Soest (1967) estimated the digestibility of ADF to be about 30% (in native material). Sample sizes of one, two and three grams were used to correspond with residual weights of in situ studies previously conducted. The samples were incubated for 6,12 and 24 h. Dry matter weight change was determined for the samples at all three times and is presented in Table 11 (expressed as a percent of the original sample weight).

Table 11. Acid detergent fiber in situ dry matter weights at 6,12 and 24 h of rumen exposure, expressed as a percent of the original sample weight.

	Time		
Sample Size	6 h	12 h	24 h
One Gram	$101^{a,x}$	$110^{b,x}$	$136^{c,x}$
Two Gram	$99^{a,y}$	$106^{a,b,x}$	$110^{b,y}$
Three Gram	$97^{a,z}$	$110^{b,x}$	$114^{b,y}$

^{a,b,c}means in the same row with different superscripts are significantly different ($P<.01$).
^{x,y,z}means in the same column with different superscripts are significantly different ($P<.01$).

Evidence of dry matter (DM) contamination can be seen in Table 11 from the percent DM recovery after incubation. Greater than 100% DM recovery was observed for all three sample sizes following 12 and 24 h of rumen incubation. For 6 h of rumen exposure the one gram sample had a one percent increase in DM recovery while the two and three gram samples had a moderate decrease of one and three percent, respectively. All of these results indicate DM contamination when the 30% digestibility of ADF is taken into consideration. There was an increase in DM contamination with increases in incubation time. Both the one and three gram samples showed significant ($P<.01$) increases in DM recovery from 6 to 12 h and from 12 to 24 h. The two gram sample had a significant ($P<.01$) increase in DM recovery from 6 to 24 h of rumen incubation. The one gram tended to have the highest recovery rates and was significantly higher at 6 and 24 h. The two and three gram samples were similar ($P>.01$) in DM recovery for 12 and 24 h. These results indicate that the smaller sample is more susceptible to contamination. This may be due to the larger surface area to sample size ratio, which allows more contact between the sample and the rumen contents.

The results of the DM contamination are magnified in the results of nitrogen contamination. The percent N increase was calculated on all three sample sizes for all three incubation times and the results are presented in Table 12.

The N contamination followed similar trends to the DM contamination. There were increases in N content of all sample sizes for all incubation times and the amount of nitrogen recovered increased with increasing rumen exposure. The one gram samples had significant ($P<.01$) increases in percent N from 6 to 12 h and from 12 to 24 h. The one gram samples also had the highest percent N for 12 and 24 h, supporting the DM results.

Table 12. Acid detergent fiber *in situ* nitrogen content change after 6,12 and 24 h of rumen exposure, expressed as percent increase in nitrogen.

	Time		
Sample Size	6 h	12 h	24 h
One Gram	32[a]	122[b]	287[c]
Two Gram	29[a]	97[a,b]	117[b]
Three Gram	34[a]	140[b]	142[b]

[a,b,c]means in the same row with different superscripts are significantly different (P<.01).

The two gram samples showed a significant (P<.01) increase in percent N from 6 to 24 h and had the lowest percent N at all times. The three gram samples increased significantly (P<.01) from 6 to 12 h but showed no significant (P>.01) change from 12 to 24 h.

The low to moderate increases in DM recovered along with the increases in percent N of the residues, indicated that there was contamination of the ADF samples. These results also indicated that the contamination was microbial in nature, because of the extreme increases in N, suggesting a contaminant high in protein (microbes). These results also agree with those of Varvikko and Lindberg (1985), who found microbial contamination of *in situ* studies of bailey straw to cause a 146% error in estimated N digestion after 12 h and 204% error after 24 h of incubation. The estimated contamination in the present study is much higher than the values found in the literature (Nocek, 1985; Blair and Cummins, 1983; Nocek and Grant, 1987). However, all of these studies were done with forages such as alfalfa hay or silage and not ADF, which may explain the

differences in results.

The results of this study indicate that there is considerable contamination of *in situ* studies of ADF. The amount of contamination found in this study would cause errors in estimated N digestion (disappearance). However, these results should be viewed and used with caution when trying to estimate the contamination of other substances, especially concentrates. These results were obtained, using a highly indigestible substrate (ADF) that is high in cellulose and was ground very fine. Nocek and Grant (1987) indicate that contamination of forages is higher than concentrates and Lathan et al. (1978) found adhesion of cellulolytic bacteria to be higher on cut edges than to surfaces not cut.

Summary

In the first experiment (I), in situ dry matter disappearance (DMD) and nitrogen disappearance (ND) was determined for raw soybeans (RAW), solvent extracted soybean meal (SBM), heat treated whole soybeans (HT), mechanically extracted soybean meal (MEX), wet extruded soybeans (TEX) and dry extruded soybeans (DEX). The results of this study showed that the length of rumen exposure did affect the DMD of all products, while it only effected the ND of RAW and SBM. As expected the unprocessed RAW had the highest DMD and ND. The DMD and ND of SBM were second highest and significantly ($P<.05$) higher than all products except RAW. The DMD of DEX was lowest and the DMD of HT, MEX and TEX were intermediate. The ND of DEX, TEX, MEX and HT were similar ($P>.05$) and were lower than that of SBM and RAW.

These results indicate that processing (heat/moisture) of protein supplements tends to decrease rumen degradability. The addition of heat or the heating of the product, due to processing, appears to be the most important factor in reducing ruminal degradability of protein. Heating to 150+°C lowered the ND, of HT, MEX, TEX and DEX as compared to SBM and RAW. The addition of moisture, in the form of steam, along with heating did not reduce ND. As was expected, the DMD and ND of the more highly processed products of DEX, TEX, MEX and HT were lower than SBM and RAW.

In the second experiment (II), the effects of time (6-12-24 h) and sample size (1-2-3 grams) on contamination of in situ studies of wheat

straw acid detergent fiber (ADF) were studied. Length of rumen exposure had a definite affect on contamination of both DM and N. Significant ($P<.01$) increases in DM recovery were observed from 6 to 12 h and 12 to 24 h on the one gram sample and from 6 to 24 h and 6 to 12 on the two and three gram samples respectively. The same results were also found for N content contamination over time.

The effect of sample size was unclear but the one gram sample had the highest degree of both DM and N contamination at all times. This indicates that the smaller sample, having a higher bag surface area to sample weight ratio, was more susceptible to contamination due to more contact of the sample with the rumen contents. Potential problems with DM and N contamination of ADF in situ studies were demonstrated with small increases in DM weights and larger increases in N content. Caution should be taken, however, when trying to use these results to estimate the contamination of other in situ studies.

Appendix

Table 1. Raw data for *in situ* dry matter and nitrogen disappearance for raw soybeans incubated for 3,6,12 and 24 hours.

Dry sample weight	Percent N*	Crude protein weight	Time in rumen	Dry residue weight	Percent N	Crude protein weight
g	%	mg	h	g	%	mg
4.58	6.75	1932	03	3.59	5.72	1283
4.58	6.75	1932	03	3.63	5.83	1323
4.58	6.75	1932	03	3.48	5.69	1238
4.58	6.75	1932	06	2.91	5.94	1080
4.58	6.75	1932	06	3.04	6.04	1148
4.58	6.75	1932	06	2.95	6.17	1138
4.67	6.75	1970	12	1.86	6.05	703
4.67	6.75	1970	12	1.87	6.17	721
4.57	6.75	1928	12	2.11	6.24	823
4.38	6.75	1848	24	0.31	2.62	164
4.38	6.75	1848	24	0.26	3.54	58
4.57	6.75	1928	24	1.31	3.69	302

*Percent nitrogen on original sample and corresponds to 42% CP.

Table 2. Raw data for in situ dry matter and nitrogen disappearance for solvent extracted soybean meal incubated for 3,6,12 and 24 hours.

Dry sample weight	Percent N*	Crude protein weight	Time in rumen	Dry residue weight	Percent N	Crude protein weight
g	%	mg	h	g	%	mg
4.57	8.25	2354	03	3.53	8.85	1953
4.57	8.25	2354	03	3.43	8.69	1863
4.25	8.25	2191	03	3.33	8.61	1792
4.55	8.25	2346	06	3.08	8.86	1706
4.55	8.25	2346	06	3.12	8.82	1720
4.55	8.25	2346	06	3.16	9.02	1781
4.62	8.25	2380	12	3.00	8.96	1680
4.62	8.25	2380	12	2.58	9.96	1606
4.62	8.25	2380	12	2.47	10.10	1559
4.43	8.25	2286	24	1.77	10.19	1127
4.43	8.25	2286	24	2.18	10.04	1368
4.43	8.25	2286	24	1.82	10.00	1138

*Percent nitrogen on original sample and corresponds to 51.5% CP.

Table 3. Raw data for *in situ* dry matter and nitrogen disappearance for dry extruded soybeans incubated for 3,6,12 and 24 hours.

Dry sample weight	Percent N*	Crude protein weight	Time in rumen	Dry residue weight	Percent N	Crude protein weight
g	%	mg	h	g	%	mg
4.50	5.65	1589	03	3.82	5.93	1416
4.50	5.65	1589	03	3.80	5.98	1420
4.50	5.65	1589	03	3.78	6.04	1427
4.48	5.65	1583	06	3.67	6.48	1486
4.48	5.65	1583	06	3.48	6.45	1403
4.48	5.65	1583	06	3.85	6.14	1477
4.50	5.65	1589	12	3.26	6.61	1347
4.48	5.65	1583	12	3.23	6.53	1318
4.48	5.65	1583	12	3.30	6.60	1361
4.42	5.65	1559	24	3.24	6.38	1292
4.42	5.65	1559	24	3.10	6.70	1298
4.43	5.65	1562	24	3.21	6.56	1316

*Percent nitrogen on original sample and corresponds to 35% CP.

Table 4. Raw data for *in situ* dry matter and nitrogen disappearance for mechanically extracted soybean meal incubated for 3,6,12 and 24 hours.

Dry sample weight	Percent N*	Crude protein weight	Time in rumen	Dry residue weight	Percent N	Crude protein weight
g	%	mg	h	g	%	mg
4.57	7.50	2140	03	3.73	8.03	1935
4.57	7.50	2140	03	3.93	7.72	1896
4.57	7.50	2140	03	3.78	7.96	1881
4.53	7.50	2125	06	3.61	8.21	1852
4.53	7.50	2125	06	3.26	8.69	1771
4.53	7.50	2125	06	3.20	8.80	1760
4.58	7.50	2148	12	2.80	9.51	1664
4.58	7.50	2148	12	3.00	9.14	1714
4.58	7.50	2148	12	3.12	8.85	1726
4.49	7.50	2102	24	2.65	9.67	1602
4.49	7.50	2102	24	2.75	9.38	1612
4.49	7.50	2102	24	2.65	9.66	1600

*Percent nitrogen on original sample and corresponds to 47% CP.

Table 5. Raw data for *in situ* dry matter and nitrogen disappearance for heat treated soybeans incubated for 3,6,12 and 24 hours.

Dry sample weight	Percent N*	Crude protein weight	Time in rumen	Dry residue weight	Percent N	Crude protein weight
g	%	mg	h	g	%	mg
4.65	6.47	1880	03	4.16	6.00	1560
4.65	6.47	1880	03	3.75	6.41	1502
4.65	6.47	1880	03	4.03	6.41	1615
4.60	6.47	1860	06	3.50	6.88	1505
4.60	6.47	1860	06	3.09	7.11	1373
4.60	6.47	1860	06	3.33	6.97	1451
4.68	6.47	1894	12	3.13	7.13	1395
4.68	6.47	1894	12	2.99	7.25	1355
4.68	6.47	1894	12	3.07	7.35	1410
4.50	6.47	1820	24	2.51	7.33	1150
4.50	6.47	1820	24	3.08	7.16	1378
4.50	6.47	1820	24	2.65	6.93	1148

*Percent nitrogen on original sample and corresponds to 40.5% CP.

Table 6. Raw data for *in situ* dry matter and nitrogen disappearance for wet extruded soybeans incubated for 3,6,12 and 24 hours.

Dry sample weight	Percent N*	Crude protein weight	Time in rumen	Dry residue weight	Percent N	Crude protein weight
g	%	mg	h	g	%	mg
4.30	8.83	2373	03	3.81	9.16	2181
4.30	8.83	2373	03	3.59	9.72	2181
4.30	8.83	2373	03	3.92	8.98	2200
4.52	8.83	2494	06	3.14	10.61	2082
4.52	8.83	2494	06	3.28	10.30	2112
4.52	8.83	2494	06	3.12	10.63	2073
4.50	8.83	2483	12	2.88	11.11	2000
4.50	8.83	2483	12	2.91	11.21	2039
4.50	8.83	2483	12	2.87	11.37	2039
4.53	8.83	2500	24	2.50	12.10	1891
4.53	8.83	2500	24	2.63	12.09	1987
4.53	8.83	2500	24	2.57	11.80	1895

*Percent nitrogen on original sample and corresponds to 55% CP.

Table 7. Percent in situ dry matter disappearance of dry extruded soybeans, heat treated soybeans, mechanically extracted soybean meal, wet extruded soybeans, solvent extracted soybean meal and raw soybeans at 3,6,12 and 24 hours of rumen exposure.

	Time			
	3	6	12	24
Feed	Percent Dry Matter Disappearance			
Dry Extruded	$15.6^{a,w,x}$	$18.2^{a,b,w}$	$27.3^{b,c,w}$	$28.0^{c,w}$
Heat Treated	$14.4^{a,w,x}$	$28.1^{b,x}$	$34.5^{b,c,w,x}$	$39.0^{c,x}$
Mechanically Extracted	$16.6^{a,w,x}$	$25.9^{b,w,x}$	$35.0^{b,c,w,x}$	$40.2^{c,x}$
Wet Extruded	$12.3^{a,w}$	$29.7^{b,x}$	$35.9^{b,c,w,x}$	$43.3^{c,x}$
Solvent Extracted	$23.2^{a,x}$	$31.4^{a,x}$	$41.9^{b,x}$	$56.6^{c,y}$
Raw Soybeans	$22.1^{a,w,x}$	$35.2^{b,x}$	$58.0^{c,y}$	$85.9^{d,z}$

^{a,b,c,d}means in the same rows with different superscripts are significantly different ($P<.01$).

^{w,x,y,z}means in the same columns with different superscripts are significantly different ($P<.01$).

Table 8. Percent in situ nitrogen disappearance of dry extruded soybeans, wet extruded soybeans, mechanically extracted soybean meal, heat treated soybeans, solvent extracted soybean meal and raw soybeans at 3,6,12 and 24 hours of rumen exposure.

	Time			
	3	6	12	24
Feed	Percent Nitrogen Disappearance			
Dry Extruded	$10.6^{a,x}$	$8.7^{a,x}$	$15.3^{a,x}$	$16.5^{a,x}$
Wet Extruded	$7.8^{a,x}$	$16.2^{a,x}$	$18.4^{a,x}$	$23.0^{a,x}$
Mechanically Extracted	$11.0^{a,x}$	$15.6^{a,x}$	$20.8^{a,x}$	$23.7^{a,x}$
Heat Treated	$17.1^{a,x,y}$	$22.4^{a,x,y}$	$26.8^{a,x}$	$32.7^{a,x,y}$
Solvent Extracted	$18.7^{a,x}$	$26.0^{a,x,y}$	$32.1^{a,b,x}$	$47.0^{b,y}$
Raw Soybeans	$33.7^{a,y}$	$61.9^{a,y}$	$61.7^{b,y}$	$90.8^{c,z}$

^{a,b,c}means in the same rows with different superscripts are significantly different ($P<.01$).

^{x,y,z}means in the same columns with different superscripts are significantly different ($P<.01$).

Table 9. Raw data for *in situ* dry matter and nitrogen change for acid detergent fiber incubated for 6,12 and 24 hours.

Dry sample weight	Percent N*	Crude protein weight	Time in rumen	Dry residue weight	Percent N	Crude protein weight
g	%	mg	h	g	%	mg
0.95	0.95	55	06	0.94	1.30	76
0.94	0.95	56	06	0.95	1.20	71
1.83	0.95	109	06	1.81	1.23	139
1.86	0.95	110	06	1.85	1.25	145
2.76	0.95	164	06	2.69	1.30	219
2.77	0.95	165	06	2.68	1.33	223
0.92	0.95	54	12	1.01	1.90	120
0.92	0.95	54	12	1.01	1.92	121
0.95	0.95	57	12	1.03	1.97	127
1.83	0.95	109	12	1.93	1.72	207
1.83	0.95	109	12	1.91	1.65	197
1.83	0.95	109	12	2.01	1.90	239
2.75	0.95	163	12	2.99	2.03	379
2.75	0.95	163	12	3.07	2.11	405
0.92	0.95	54	24	1.25	2.57	201
0.92	0.95	54	24	1.27	2.77	220
1.83	0.95	109	24	2.04	1.85	236
1.83	0.95	109	24	1.96	1.71	209
1.83	0.95	109	24	2.07	2.05	265
2.75	0.95	163	24	3.18	2.08	413
2.75	0.95	163	24	3.10	1.97	382
2.75	0.95	163	24	3.13	2.00	391

*Percent nitrogen on original sample and corresponds to 6% CP.

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